aminase [32]) raised the possibility that this ara-A analog or one of its derivatives might be pharmacologically superior to ara-A, capable of producing a stable and more prolonged antiviral effect. We have therefore pursued additional work with cyclaradine and its more lipophilic 5'-methoxyacetic acid ester (Fig. 1) in vivo, and we have compared the therapeutic efficacy of these new agents with that of ara-A in the systemic treatment of experimentally induced HSV-1 encephalitis in mice.

MATERIALS AND METHODS

Virus. The HS-123 strain of HSV-1, originally obtained from Robert D. Francis, University of Alabama Medical Center, Birmingham, was used in these studies. Virus was passaged intracerebrally (i.c.) in Swiss mice, and a stock virus pool was prepared as a 10% mouse brain suspension in Hanks balanced salt solution containing 5% fetal bovine serum.

Animals and virus inoculation. Random-bred Swiss mice (female), weighing 18 to 22 g, were obtained from Charles River Breeding Laboratories, Inc., Wilmington, Mass. Animals were inoculated either intraperitoneally (i.p.) with 6.4 50% lethal doses (LD_{50}) of virus (0.3 ml of the appropriate dilution of stock virus suspension) or i.c. with 10 or 32 LD_{50} of virus (0.03 ml of the appropriate dilution of stock virus suspension) and observed for 21 days after virus inoculation.

Antiviral drugs. Cyclaradine was prepared by methods described earlier (32). The synthesis of cyclaradine-5'-methoxyacetate will be reported elsewhere (Vince et al., manuscript in preparation). Micronized ara-A was generously supplied by the Warner-Lambert Pharmaceutical Research Div., Warner-Lambert Co., Ann Arbor, Mich. For injections, drugs were suspended in 0.9% saline solution containing 0.3% hydroxypropyl cellulose.

Antiviral evaluation in vivo. Drugs were administered to mice i.p. once daily for 7 days beginning 4 h after virus inoculation. Groups of 10 infected and 5 to 10 uninfected animals were treated with each drug dose level (uninfected, drug-treated mice served as toxicity controls). Twenty virus-infected, untreated mice served as the virus control group. In addition, a group of uninfected, untreated mice was held as a normal control group. Results were expressed in terms of percent survivors in the virus-infected, drug-treated groups compared with percent survivors observed in the virus-infected control (untreated) group or in terms of the mean survival time of mice dying on or before day 21 postinfection in the drug-treated groups compared with that observed in the untreated control group.

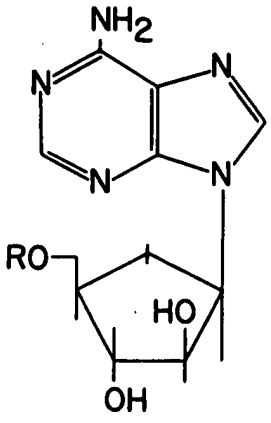

FIG. 1. Chemical structure of cyclaradine (R = H) and cyclaradine-5'-methoxyacetate (R = $-COCH_2OCH_3$). The structure depicts one enantiomer of the racemic compound actually obtained.

Statistical evaluation. Data were subjected to the appropriate statistical test for the determination of the levels of significance of differences observed. To compare the mortality of untreated and drug-treated mice, we evaluated the data by the chi-square test with the Yates correction. To compare the differences in mean survival time observed between virus-infected control (untreated) and virus-infected, drug-treated mice, we evaluated the data by the Student t test. A P value of <0.05 was considered indicative of a statistically significant difference.

RESULTS

Efficacy of cyclaradine in mice inoculated i.p. with HSV-1. The significant therapeutic effect of cyclaradine against lethal HSV-1 infections in mice, after i.p. inoculation of virus, was readily demonstrated (Table 1). Cyclaradine was found to be as effective as ara-A in reducing mortality in the virus-infected animals. Whereas 95% of the virus-infected control (untreated) mice died, with a mean survival time of 8.4 days, systemic treatment with ara-A reduced the mortality to 0 to 10% when the drug was administered i.p. at nontoxic doses once a day for 7 days starting 4 h after virus inoculation. Likewise, cyclaradine was also significantly effective ($P < 0.0005$) in decreasing the mortality to 0 to 10% when administered i.p. to infected animals at nontoxic doses of between 112.5 and 450 mg/kg per day on the same treatment schedule. Cyclaradine at a dose level of 900 mg/kg per day (once daily for 7 days) was apparently not lethally toxic for uninfected animals, but failure to gain weight was noted. The same dose level proved to be toxic for HSV-infected mice, with a mean weight loss of 1.7 g. The mean survival time for HSV-infected mice was significantly shortened ($P < 0.005$) to 4.3 days. The LD_{10} for ara-A on the same schedule was ca. 600 mg/kg per day.

Efficacy of cyclaradine and its 5'-methoxyacetic acid ester in mice inoculated i.c. with HSV-1. We compared the therapeutic activities of ara-A and cyclaradine at equimolar dose levels against lethal HSV-1 infections in mice after i.c. inoculation with 32 LD_{50} of virus. This is an extremely severe test for any antiherpesvirus drug, including ara-A. All of the virus-infected control (untreated) mice died with a rapidly progressive and fulminating herpesvirus encephalitis, with a